SYNOPSIS

PhD in Zoology

Exploring the potential of *Rhizoclonium hieroglyphicum* as a biosorbent for the removal of Reactive Orange 107 and its toxicological implications and reusability

By

A V Swathilakshmi

(19PHZOF005)

Supervisor

Dr. M. Poonkothai

Associate Professor

Department of Zoology

Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore – 641043

May 2024

Introduction

Rapid industrialization, urban expansion and technological advancements leads to the generation of intricate and hazardous forms of pollution (Khan *et al*., 2022). The exponential growth of textile industry has led to the widespread use of dyes across various sectors, which has resulted in the discharge of untreated dyeing effluent or waste water into the ecosystem causing pollution. The discharge of effluent containing complex mixture of chemicals and dyes severely affects the aquatic bodies and make them unsuitable for the biota (Awwad *et al*., 2020). Reactive dyes namely Reactive Orange 107, Reactive Red 239, Reactive Yellow 145 and Reactive Blue 19 are extensively used in textile industry because of its soluble nature and can easily bind to the fibres by the formation of covalent bonds. In this study Reactive Orange 107 was selected as the candidate dye because of its wide use in various industries. High exposure to this dye may lead to vomiting, diarrhoea, cardiovascular shock, gastrointestinal discomfort, teratogenesis, cancer and can also trigger allergic reactions in human beings (Sarojini *et al*., 2023). In order to eradicate hazardous contaminants from textile wastewater, a plethora of physico-chemical treatment methods such as flocculation, precipitation, membrane filtration, coagulation, ion-exchange and adsorption were often practiced. But these methods remain unfeasible for industrial practice due to their high cost and enormous sludge generation (Deokar and Sabale, 2014). To minimize the toxicological effect of dyes without disturbing the ecosystem, bioremediation has gained attention because of its low toxic potential, ease of production and cost effectiveness. Several biologicals such as bacteria, fungi, algae and adsorbents have been reported for the removal of color from the textile dyeing effluent. Among the biologicals, algae have received a lot of attention because of its immense availability and for their propensity to use the pollutants as a source of nourishment and destroy them enzymatically. Unlike plants, algae do not require oxygen to perform photosynthetic activity, so they fix carbon - dioxide in water systems and also develop a tolerance for contaminants. They also have an excellent cell wall features for biosorption of dyes due to their strong binding affinity, complexation and electrostatic attraction. They possess polyphosphate bodies which aid to store the toxicants in the storage pool and also help in detoxification mechanism. The functional groups linked to the algal cell surface are supposed to play a key role in the removal of colour from the textile waste water (Stenico *et al*., 2012; Donmez and Akshu, 2002). *Rhizoclonium* sp and *Cladophora* sp are filamentous algae which belongs to the family Cladophoraceae, are prevalent in freshwater environment. They exhibit rapid proliferation under specific conditions and forms dense mat particularly in nutrient-rich waters. The

excessive presence of nutrients, notably nitrogen and phosphorus originating from sources like agricultural runoff, wastewater discharge or human activities fosters the algal growth. While the algae aids in pollutant removal, its overabundance poses challenges by impeding human activities in water bodies. These issues encompass reduced flow rates, obstruction of outfalls, pump intakes and the unattractive appearance. Despite these hindrances, it is essential to acknowledge the efficiency of algae in pollutant removal (Robson *et al*., 1976).

With this background information, the present study was aimed to harness the potential of algae as a biosorbent for the removal of RO107 from aqueous solutions under optimized conditions to assess its toxicological assessments and reuse potential. The objectives of the present study are as follows.

- To determine the efficacy of the algae for the decolorization of selected reactive dyes.
- To identify and characterize the selected biosorbent
- To optimize the process parameters for dye decolorization using Response Surface Methodology (RSM), Random Forest (RF) and Artificial neural network (ANN)
- To assess the adsorption mechanism and adsorbent-adsorbate interactions of dye onto the biosorbent
- To assess the dye elution and regeneration efficiency of the biosorbent
- To evaluate the physico-chemical and irrigational quality of untreated and treated dye solutions.
- To assess the toxicity potential of untreated and treated dye solutions using various biologicals.
- To analyze the reuse potential of dye desorbed algae and treated dye solution for composting and dyeing fabrics.

Materials and Methods

Adsorbate preparation

Textile dyes such as Reactive Orange 107 (RO107), Reactive Red 239 (RR239), Reactive Blue 19 (RB19) and Reactive Yellow 145 (RY145) were acquired from the local dyeing industries, Perundurai, Tamil Nadu, India. To prepare the stock solution, 1g of each dye was dissolved in 1 L of water and the desired concentrations were achieved through consequent dilutions.

Collection of the biosorbents

The algae for the study namely *Cladophora* sp and *Rhizoclonium* sp were collected from fresh water pond, Sulur (Latitude – 10.911226°, Longitude – 77.132494°), Coimbatore and Unjapalayam, Erode (Latitude-11.5917°, Longitude - 77.7107°), Tamil Nadu, India. The algae were washed in running tap water and further rinsed with distilled water to remove the dirt adhered on it. The algae were shade dried, crushed, sieved, powdered and kept in air tight container for further use.

Preliminary screening of the dyes for decolorization using the biosorbents

Batch adsorption experiments were conducted to assess the decolorization of selected dyes from aqueous solutions using the selected algae. In a series of 1L Erlenmeyer flasks, varying concentrations of dyes (10-1000 mg/L) were added with diverse amounts of biosorbents (10-1000 mg/L) and incubated at different time intervals (12-108 hours). The pH of the solutions was adjusted within the range of 2-12 using 1N HCl or 1N NaOH and the temperature was varied between 25-45°C while maintaining other conditions constant. The experiment was conducted in triplicates and the dye solutions without algae served as control. After each incubation period, the contents were filtered using Whatman filter paper no 1 and subsequently measured using UV–vis spectrophotometer at the required wavelength. The percentage of decolorization was calculated using the given formula,

$$\text{Decolorization (\%)} = \frac{A_i - A_t}{A_i} \text{ x } 100 - - - - - (1)$$

Where A_i is the initial absorbance and A_t is the final absorbance at incubation time t (Kaur *et al.,* 2015). The decolourisation percentage of selected dyes by the algae were assessed and the alga which exhibited maximum decolorization was selected for further studies.

Identification and Characterization of the selected alga

The selected alga was submitted for identification in Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The surface properties of the biosorbent namely surface area, pore diameter, pore size and porosity were characterized by Brunauer-Emmett-Teller analyser (Belsorp – Mini II, USA). The particle size of the biosorbent was analysed using malvern zetasizer vr 7.11. The moisture content was determined using moisture analyser (Wensar HMB50H). Bulk density of the biosorbent was determined

using Archimedes' principle. The point of zero charge (pHpzc) was determined using a batch equilibrium method (Jawad *et al*., 2017).

Optimization of process parameters for dye decolorization using regression analysis

To determine the most effective conditions for the optimization of RO107 removal, three regression models namely Response Surface Methodology, Random Forest and Artificial Neural Network were performed, compared and evaluated.

Response Surface Methodology

Response Surface Methodology (RSM) is a mathematical and statistical approach for optimizing process variables to obtain the best result with least number of runs. RSM is more efficient for designing and optimizing experiments when combined with Box-Behnken Design (BBD). The impact of independent variables namely initial dye concentration (A), biosorbent concentration (B), pH (C) and incubation time (D) were evaluated and further confirmation was carried out by batch adsorption studies. It was performed by varying different concentrations of dye (100 - 500mg/L) and biosorbent (100 - 900mg/L), incubation time (24 - 120 hours) and pH (4 - 12) was adjusted with 0.1N HCl and 0.1N NaOH while the temperature (25ºC) remains constant. To assess the impact of independent variables on RO107 removal, a total of 29 trials were used in this study. Regression analysis was performed by employing a second-order polynomial equation to unveil the interaction among input variables in relation to the removal of RO107. The adequacy of the design model was evaluated using analysis of variance (ANOVA). The Fisher variation ratio (F value) and probability value (P value) were used to assess the relevance of the model (significant probability value). Also, the coefficient of determination (R^2) was used to determine the correlation between the experimental and predicted values. 3D response surface plot visualisation was employed to analyse the interactive effect of different variables on the dye decolorization (Kumar *et al*., 2019).

Random forest

Random Forest (RF) is a non-parametric ensemble machine learning technique combines an extensive set of unpruned decision trees to generate the desired output. To implement the Random Forest (RF) method Python version 3.6.8 were utilized. The Random Forest Regressor function was employed with default configurations. To assess dye decolorization, the RSM dataset was divided into training, testing and validating sets. To evaluate the performance of the RF algorithm, the trees are cross-validated and appropriate

regression metrics such as Mean Absolute Error (MAE), Mean Squared Error (MSE), Root Mean Squared Error (RMSE) and R-squared (R^2) are employed (Soares *et al*., 2020).

Artificial Neural Network

Artificial Neural Network (ANN) is a non-linear mathematical model inspired by the simulation of biological nervous system, serve as effective tool for mapping relationship between input and output parameters. ANN model was developed by employing Multilayer Perceptron (MLP) network to predict RO107 removal using Python 3.6.8. In the present study, feed forward multilayered perceptron consisting of three layers namely input layer of neuron (independent variables), a number of hidden layers and output layer (dependent variables) combined with activation function (Sigmoid) were employed. The RSM dataset was divided into training, validation and testing in the ratio of 70%:15%:15%. To quantify the model's forecasting capacity, Mean Square Error (MSE), Absolute Mean Error (MAE) and Coefficient of Determination (R^2), Root Mean Squared Error (RMSE) were assessed (Vinayagam *et al*., 2022).

Adsorption isotherms, kinetics and thermodynamic studies

Adsorption isotherms namely Langmuir, Freundlich and Temkin models are important for determining the affinity between the biosorbent and the adsorbate for understanding the removal mechanisms in large-scale applications. Adsorption kinetics (Pseudo first order, Pseudo second order and Intra particle diffusion) gives insight into the reaction pathway and rate limiting steps between adsorbate and the biosorbent. The effect of change in temperature on the adsorption process was performed using thermodynamic parameters namely Gibbs free energy(ΔG^o), Enthalpy(ΔH^o) and Entropy(ΔS^o) respectively (Lata *et al*., 2007).

Analytical studies

To assess the changes in adsorption spectra, the untreated and treated dye solutions were subjected to UV–Visible spectrophotometer (Shimadzu / 206 – 26300 - 48, Japan) at 200 – 800 nm. Fourier Transform Infrared Spectroscopy (FT-IR) (Shimadzu (Miracle 10), Japan) was performed for dye loaded and unloaded alga at 4000 – 400 cm^{-1} to determine the shifts in the functional groups involved during the process of dye decolorization. The surface morphology of the dye loaded and unloaded alga was analyzed using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) (TESCAN — MIRA3 XMU, Czech Republic). The X-ray diffractometry (XRD) was used to determine the nature of dye unloaded and loaded alga scanned

at the ratio of 3°/min over the range of 20°- 80° (2θ) (Philips PW-3710, England) (Oualid *et al*., 2020).

Desorption and Regeneration studies

To determine the desorption efficiency, dye-loaded biosorbent (500 mg/L) was added to 100mL Erlenmeyer flask containing eluting agents (0.1M NaOH, 0.1M HNO_3, 0.1M HCl and 0.1M KOH) separately and agitated at 200 rpm for 3 hours at room temperature. The experiment was conducted in three consecutive cycles at 25°C. The ratio between the amount of dye desorbed and the amount of dye adsorbed was used to determine the desorption efficiency of the biosorbent. In the regeneration experiment, the dye-loaded biosorbent which was eluted using the desorbing agents was thoroughly washed thrice with distilled water to remove any traces of dye and further mixed with the dye solution for the next adsorption cycle and further the percent decolourisation was calculated (Indah *et al*., 2018).

Characterization of physico-chemical parameters in the untreated and treated dye solutions

To determine the quality of untreated and treated dye solutions, the physical parameters (colour, odour, pH, electrical conductivity, temperature, total suspended solids (TSS), total dissolved solids (TDS) and total solids (TS)) and chemical parameters (salinity, chloride, dissolved oxygen, biological oxygen demand, chemical oxygen demand, total hardness, alkalinity, calcium, magnesium, sulphate, phosphate, nitrate, fluoride, sodium and potassium were analyzed as per the standard procedure (APHA, 2012).

Suitability analysis of untreated and treated dye solutions for irrigation purpose

In recent years, the increasing use of wastewater for irrigation underscores the need to assess the irrigation quality of wastewater. Therefore, the suitability of wastewater for irrigation purposes is assessed based on the parameters namely the Sodium adsorption ratio (SAR), Sodium soluble percentage (SSP), Kelly ratio (KR), Magnesium ratio (MR), Residual sodium carbonate (RSC), Permeability index (PI), Corrosivity ratio (CR) and Potential salinity (PS) (Saber *et al*., 2022).

Toxicological assessment of untreated and treated dye solutions using various biological models

Toxicity assessment using *Vigna radiata*

The study was conducted using the seeds of *Vigna radiata* (10 seeds) at room temperature by watering regularly with tap water (control), untreated and treated dye solutions for 7 days. At the end of the 7th day, germination percentage, plumule and radicle length, vigour index, germination rate index, germination speed, germination energy, tolerance index, phytotoxicity percentage and phytotoxicity index were calculated (Vaithiyanathan and Sundaramurthy, 2017)

Toxicity assessment using *Allium cepa*

Allium cepa bulbs were grown for five days at room temperature in glass beakers containing 200mL of tap water (control), untreated and treated dye solutions. The dye solutions and tap water were changed every 24 hours. At the end of the fifth day, the germinated bulbs were removed and the root meristem of each treatment was cut into 2 cm lengths, cleansed with distilled water and exposed to Carnoy's solution for 5 minutes. The fixed root tips were hydrolyzed in 1N HCl at 60°C for 5 minutes to soften the tissue. The root tips were squashed, covered with a coverslip and stained with acetoorecin for 15 minutes. Under a light microscope, the cells were observed to assess different mitotic phases and chromosomal abnormalities in each treatment. The root growth, root length, mitotic inhibition, mitotic depression and the chromosomal aberrations were evaluated (Prasad and Rao, 2013).

Toxicity assessment using *Artemia salina*

The eggs of Brine shrimp (*Artemia salina*) were incubated overnight for hatching in a trough containing saline water with ambient light at laboratory. After 24 hours newly hatched larva numbering 20 was selected and added into saline water (90mL) amended with untreated and treated dye solutions separately. For every 3hrs time interval upto 24 hours the mortality of *A. salina* was recorded (Eshghi and Kashi, 2022).

Toxicity assessment using *Catla catla*

The fingerlings of *Catla catla* were procured from Aliyar Fish Form, Tamil Nadu Fisheries Development Corporation Limited, Pollachi and transported to the laboratory. The collected fishes were acclimatized for 15 days in laboratory condition and fed with rice bran. Ten fishes were introduced into plastic trough (5L capacity) containing tap water (T_1) which served as control, untreated (T_2) and treated (T_3) dye solution separately. Each treatment was

repeated thrice and maintained under 12:12 light-dark photoperiod. The experiment was conducted for a period 30 days in laboratory conditions.

At the end of 30th day, the fishes from each treatment were subjected to haematological (White Blood Corpuscles, Red Blood Corpuscles, Haemoglobin, Packed Cell Volume, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration), biochemical (Serum glucose, serum protein and serum cholesterol) and enzymological (Aspartate aminotransferase, Alanine Transaminase and Lactate dehydrogenase) analyses. Histological examination was performed on the gill, liver, and kidney tissues of the fishes exposed to the treatments (Paramar and Shah, 2017).

Reutilization of treated dye solution for dyeing fabrics

In order to control water pollution, recycling of waste water is the need of hour. Hence an attempt has been made to utilize the treated dye solution for dyeing fabrics using optimum dye concentration (300mg/L). The parameters such as fabric weight, thickness, strength, elongation, stiffness, colour strength and colour fastness were analysed in the fabric dyed using treated dye solution (Kandasamy *et al*., 2021).

Preparation of compost using dye desorbed alga

The dye desorbed alga was repurposed as organic waste through composting in a laboratory scale. The organic waste materials consisting of dye-desorbed algal powder, garden waste and cow dung in the ratio of 2:1:2 were assessed and allowed to decompose for a period of 60 days maintaining the moisture levels through regular watering. To manage heat generated during decomposition, the composting materials were periodically sprinkled with water and the compost was harvested and analysed for various physico-chemical parameters such as pH, electrical conductivity, organic carbon and macronutrients (nitrogen, phosphorus and potassium) (Khan *et al*., 2019).

Decolorization of textile effluent using the selected alga

The real textile industrial effluent was collected from the local dyeing industry, Perundurai, Tamil Nadu, India. The biosorbent was added into the effluent under optimized conditions and the decolorization percentage was determined (Naggar *et al*., 2020).

Results and Discussion

Preliminary screening of the dye and biosorbent using batch decolorization study

The results of the preliminary screening revealed that 87% and 96% color was removed in RO107 using *Cladophora* sp and *Rhizoclonium* sp at 100 mg/L of dye concentration amended with 200 mg/L of biosorbent at pH 8 for 72 hours of incubation and 300 mg/L of dye

concentration amended with 500 mg/L of biosorbent at pH 9 for 72 hours of incubation respectively. The color removal ranged from 90 – 60 % in the dyes namely RR239, RB19, RY145 exposed to *Cladophora* sp and *Rhizoclonium* sp respectively. Thus, it was evident from the preliminary screening of dyes and biosorbent that maximum decolourisation percentage was recorded for reactive orange 107 using *Rhizoclonium* sp and further studies was carried out using the same as candidate dye and biosorbent.

Identification and characterization of the selected alga

The alga which exhibited maximum dye decolorization was identified microscopically as *Rhizoclonium hieroglyphicum* and the voucher specimen number is BSI/ SRC/5/23/2020/TECH/841. *Rhizoclonium hieroglyphicum* a long, unbranched filamentous fresh water green alga belongs to the family *Cladophoraceae* possess short, lateral rhizoids through which it adheres to the substratum. The cells are flexuous, cylindrical and multinucleated with thin cell wall which is rich in polysaccharides. It is found in both marine and freshwater environments and commonly seen in brackish waters. The thallus was dark green, flaccid with soft texture and often occurs as a floating wooly dense mat on the substrate.

The BET surface area of *Rhizoclonium hieroglyphicum* is 3. 5528 m^2g^{-1} with total pore volume of 0.0737 cm^2g^{-1} whereas the average pore diameter is 19.56nm which is in the mesoporous scale (2 – 50nm) as confirmed by International Union of Pure and Applied Chemistry (IUPAC) classification of pores by size. It is evident that the Nitrogen adsorption-desorption isotherm pattern in *Rhizoclonium hieroglyphicum* fits the type IV isotherm attained with mesoporous solids. The average size of the biosorbent was recorded as 851.7nm.

The point zero charge value refers to the pH at which the net surface charge of the biosorbent is zero. Therefore, for the stronger electrostatic interaction, when the pH is higher than the point zero charge value, cationic solutes are better adsorbed whereas the adsorption of anionic solutes is preferred when the pH is lower than the point zero charge value. The pHpzc of *Rhizoclonium hieroglyphicum* was recorded at pH 6 indicating the positive surface charge of the biosorbent. Thus, acidic pH of the biosorbent is beneficial for anionic dye adsorption.

The bulk density of the biosorbent is measured at 0.275 g/cm^3. According to Jawad *et al*., (2016s), a low bulk density implies the porosity of the biosorbent is feasible for adsorption.

The moisture content in *Rhizoclonium hieroglyphicum* was recorded at 19.52% which coincides with Wang *et al*., (2019) who stated that high moisture content does not favour adsorption whereas lower moisture content enhances the adsorption capacity of biosorbents.

Optimization of process parameters for dye decolorization using regression analyses

Response surface methodology (RSM)

The decolorization of RO107 efficiency by *R. heiroglyphicum* was evaluated using BBD to optimize the operating conditions. In the present design model, a total of 29 experiments were attained randomLy to optimize the process variables for maximum decolorization of RO107. The rational of the model designed was proved using the coefficient of determination (R^2) values, where the predicted R^2 (0.9105) was in acceptable agreement with the adjusted R^2 (0.9672) thereby signifying the fact that the model could be successfully utilized for the perfect design space. The coefficient of determination (R^2) which is expressed as the fit of the model was observed to be 0. 9836 for RO107 decolorization thereby indicating that the prediction of experimental data is satisfactory. The adequate precision value (>4) of the study was higher (23.958) thereby indicating the usefulness and reliability of the developed model which can control the design space for the experimental setup. The coefficient of variation (CV) value 2.5% (desirable range < 10%) depicted low deviation between the experimental and predicted responses which specifies the high degree of precision and executed experiments.

The diagnostic plots reveals that the residuals were scattered around the straight diagonal line, close to the diagonal line, and the residuals were between the two red dashed lines, thereby demonstrating the accuracy of the model. Perturbation plot identifies the most influential factors affecting the response. The curve exhibited a smooth and symmetrical behavior around the central level of each variable indicating a good impact in dye decolorization. Box-Cox plot with optimal value (λ) =1 is associated with model design value suggested no transformation of the model, proving that the data is normally distributed and predicted. The histogram represents a bell-shaped curve centered around zero indicating the model is significant. Three-dimensional and contour plots were employed to examine the interaction among process variables and the circular or elliptical plots illustrates the significance of the model. In this study, interactions of the contour plot among all variables exhibits an elliptical form depicting the significance of the model.

Random Forest (RF) and Artificial neural network (ANN)

The data is divided into training, testing, and validation sets of RF and ANN. These practices ensure that the model is trained on relevant data, tested for performance and optimized for generalization. The actual vs predicted value of RF and ANN are closely match the actual values and there is no evidence for significant prediction errors or outliers. The residual

histogram of RF and ANN are normally distributed with a symmetric bell-shaped curve centred around zero indicating a well-behaved regression model.

Comparison of RSM, RF and ANN model

The RSM and Random Forest models demonstrated consistent and strong predictive performance, with low errors and high R^2 values, resulting in an expected value of 97.43 and 97.244, respectively. These models have proven their effectiveness in accurately capturing and predicting the decolourisation efficiency, contributing to a comprehensive understanding of the underlying factors driving the process. However, it's important to note that the ANN model exhibited high error values, low R^2 and a weaker model fit. This discrepancy may be attributed to the activation functions, which impacted the model ability to generalize the data effectively. From the regression analysis (RSM, RF and ANN) it is concluded that maximum decolorization (97%) was observed at optimum conditions of pH 9 at 500 mg/L biosorbent amended with 300 mg/L dye concentration at 72 hours of incubation time.

Adsorption isotherms, kinetics and thermodynamic studies

The results of isotherm data suited well with Freundlich isotherm than Langmuir and Temkin isotherm models thereby suggesting the adsorption process occurs on heterogeneous surface of the biosorbent. The value of 1/n indicates the type of isotherm to be irreversible if 1/n equal to 0 or favourable if 1/n less than1 or unfavourable if 1/n greater than 1. The 1/n value (0.1369) for Freundlich isotherm lies within 1 thereby reflects the heterogenous adsorption of dye onto the biosorbent. The results of adsorption kinetics revealed that the R^2 value of pseudo second order kinetic model was 0.9994 which proves to be a better fit than pseudo first order kinetic model. Thus, kinetic study provides valuable insights into the process of adsorption, which can be useful for designing and optimizing dye removal from wastewater or other environmental applications. Since the pseudo first order and pseudo second order kinetic models cannot recognize the diffusion mechanism, the kinetic data were further evaluated to identify the steps involved in the adsorption process through Weber Morris intraparticle diffusion. Deviation from the origin in the graph noted the linear line does not pass through the origin thereby depicting the intraparticle diffusion is not the only rate-limiting step in the adsorption process. The negative value for Gibbs free energy and positive value of entropy and enthalpy suggested that the biosorption is spontaneous, feasible and endothermic in nature.

Analytical studies

The absorption peak of untreated dye solution was observed at 420nm whereas in the treated dye solution the peak was shifted to 251nm indicating the decolourisation of RO107. FT-IR analysis helps in elucidating the possible interaction between the dye and the functional

groups present on the surface of the alga. The shifting and intensification of peaks indicates the involvement of biosorbent in the removal of dye. FT-IR spectrum before adsorption of RO107 revealed the presence of functional groups namely hydroxyl and amino groups on the algal surface which were the main active binding sites responsible for the adsorption of RO107. In XRD analysis, the presence of broad peaks in the spectrum of dye unloaded biosorbent may be attributed to a heterogeneous complex matrix composed of various biomolecules whereas the biosorbent after adsorption of RO107 reveals the alteration in structure which was due to the adsorption of dye molecules.

Scanning electron micrograph of the dye unloaded alga had numerous pores, linear grooves and protrusions whereas the dye gets entrapped on to the surface of the biosorbent. The EDX spectrum of the dye unloaded alga revealed major elements namely carbon, oxygen, magnesium, silica, sulphur, potassium, calcium, iron and copper whereas in dye loaded alga, the elements were increased and new elements are formed indicating the interaction of dye with biosorbent.

Desorption and regeneration studies

The maximum desorption was attained using 0.1M NaOH (88%) as eluent at 1st cycle and thereafter a decrease was noticed in consecutive cycles. Similar trend was observed in the other eluents (KOH, HNO_3 & HCl). The decline of desorption in subsequent cycles is attributed to the inability of adsorbate molecules to completely desorb from the binding sites of biosorbent.When it pertains to regeneration efficiency, decolourisation percentage was 81% in the 1^{st} cycle where it declines in the 3^{rd} cycle. This might be due to inadequate desorption of the certain adsorbate molecules from the biosorbent binding sites and absence of unoccupied sites on the surface of biosorbent.

Characterization of physical and chemical parameters in the untreated and treated dye solutions

The results of physico-chemical characterisation which revealed that the untreated dye solution exceeded the limits prescribed by BIS for inland surface water whereas values of phosphate, nitrate, calcium and total suspended solids exceeded the limits and other parameters were within the limits prescribed by BIS for treated dye solution.

Suitability analyses of untreated and treated dye solutions for irrigation purpose

The assessment of both untreated and treated dye solutions using the irrigation water quality index revealed that the discharge of the treated dye solution does not pose a potential threat to the ecosystem and is suitable for irrigation, despite the permeability index falling into the doubtful category and the potential salinity showing slightly unsatisfactory results.

Toxicological assessment of untreated and treated dye solutions using various biological models

Toxicity assessment using *Vigna radiata*

The seedlings exposed to untreated dye solution exhibited least germination percentage, radical and plumule length, germination rate index, germination speed, germination energy, and vigor index when compared with the control whereas the seedlings exposed to treated dye solution showcased average radical and plumule length, germination percentage, germination rate index, germination speed, energy, vigor index as compared with the control. The tolerance index was found to be higher in treated dye solution and low tolerance index was found in untreated dye solution. The phytotoxicity percentage was found to higher in untreated solution and low in treated dye solution. As far as phytotoxicity index is concerned the value should be between 0 and 1. If the value exceeds 1 it indicates the toxic or negative effect of the pollutant. whereas the value below 1 implies the non toxic or positive effect on the seeds. The results of the present study indicates that the phytotoxicity index was 0.22 which falls below 1 thereby representing the non-toxic nature of the treated dye solution to the test plant. The findings of the phytotoxicity study showcased the invigorating nontoxic effects of treated dye solution

Toxicity assessment using *Allium cepa*

The cytogenotoxicity was carried out using *Allium cepa* to identify the effect of untreated and treated dye solution through chromosomal aberrations and compared with control (tap water). *Allium cepa* grown with treated dye solution (T_3) showed increased root growth, root length and mitotic index and decreased mitotic inhibition, chromosomal aberration when compared with untreated dye solution. The mitotic inhibition and chromosomal aberration were high and the root length, root growth and mitotic index were least in *A. cepa* exposed to untreated dye solution.

The results revealed that onion root tips exposed to untreated dye solutions exhibited various chromosomal aberrations namely disturbed and sticky chain metaphase, binucleate cells, vagrant Chromosome, chromosome bridge, early anaphase with multiple bridges, sticky metaphase with laggard chromosome, metaphase with adherence and chromosomal fragment which showcases the toxicity of untreated dye solution. The onion root tips exposed to control (tap water) displayed normal stages of mitosis (prophase, metaphase, anaphase and telophase) whereas treated dye solution has slight aberrations and the normal mitotic divisions such as normal prophase, metaphase, anaphase and telophase were observed proves the nontoxic nature.

Toxicity assessment using *Artemia salina*

Artemia salina larva exposed to untreated dye solution showcased 100% mortality whereas the *A. salina* larvae exposed to treated dye solution exhibited a mortality of 76% at 24 hours of exposure. Light microscopy images of *A. salina* larvae exposed to untreated dye solution revealed dye accumulation in the gut region whereas the larvae exposed to saline water and treated dye solution showed normal indicating its non-toxic nature.

Toxicity assessment using *Catla catla*

The behavioural characteristic of fish exposed to untreated dye solution (T_2) showed mucus secretion, uncoordinated movement, loss of balancing, surfacing, loss of buoyancy, skin discoloration, uncontrolled jerky movements, hitting against the wall of the trough whereas normal swimming, vision and proper body motion was observed when exposed to control and treated dye solution indicating its tolerance level and depicting the reduction in the toxicity of dye. Growth parameters namely length and weight of the fingerlings were normal in control and in treated dye solution whereas it was reduced in fishes exposed to untreated dye solution. The mortality of fishes was high in untreated dye solution (83.33%) whereas the rate was lowered in treated dye solution (46.66%) and tap water (13.33%).

Fishes exposed to untreated dye solution showed reduction in RBC, PCV, Hb, MCHC, serum protein and serum cholesterol whereas the WBC, MCV, MCH, serum glucose, AST, ALT and LDH levels were elevated indicating the toxicity of the dye. No such reduction and alterations were observed in the fishes exposed to control and treated dye solution indicating the non-toxic nature.

Histological observation on the gills of control fishes showed normal architecture of gill filaments whereas untreated fishes showed necrosis, extensive fusion and mucus secretion, vasodilation and hypertrophy in primary gill lamellae, aneurysm and degeneration of epithelium. However, the fishes exposed to treated dye solution did not show any deformities in the gills. The histology of liver in control fishes showed normal hepatocytes arrangement whereas fishes exposed to untreated dye solution showed dilated sinusoids, blood congestion, sinusoidal congestion, vacuolization, hypertrophy nucleus and necrosis. No remarkable changes were observed in the hepatocytes of fishes exposed to treated dye solution. The histology of kidney in control fishes showed well characterised glomeruli and renal tubules whereas fishes exposed to untreated dye solution showed haemocytic infiltration, glomerulopathy, shrunken glomeruli, degeneration of inner epithelial layer of renal tubule and necrosis. The glomeruli were regenerated, normal physiology of bowman's capsule and renal

tubules in the fishes exposed to treated dye solution indicates the non-toxic nature of treated dye solution.

Reutilization of treated dye solution for dyeing fabrics

The fabric weight, thickness, strength and elongation of fabric in dye treated solution is on par with fabric dyed with tap water with optimum dye concentration. The colour fastness parameters namely crocking, sunlight and pressing did not alter the colour change of the fabric exposed to treated dye solution than control fabric. Thus, the treated dye solution can be reused for dyeing the fabric instead of releasing it into environment which provides a green approach.

Preparation of compost using dye desorbed alga

The integration of dye desorbed algae for composting facilitates the transformation of waste into a valuable end product. The algal compost was abundant in NPK and carbon contents and act as an fertilizer and also as a soil conditioner.

Decolourisation of textile dyeing effluent using the candidate alga

The textile dyeing effluent inoculated with *R. hieroglyphicum* recorded 88% colour removal at optimal conditions suggesting a substantial role for biosorbent in textile industries.

Conclusion

The findings of the present study proves that *R.hieroglyphicum* is a promising and effective biosorbent for the decolorization of Reactive Orange 107. The optimization of process parameters through regression analysis resulted in significant decolorization efficiency with a maximum removal of 97%. Equilibrium studies substantiated that the experimental data was fitted to the Freundlich isotherm and pseudo second order kinetic model thereby indicating strong adsorption affinity between the dye and biosorbent. Thermodynamic studies exhibit the feasibility, spontaneity and endothermic nature of RO107 decolorization by *R. hieroglyphicum*. Analytical studies validate the affirmative interaction between the dye and biosorbent. The desorbed biosorbent can be reused without any loss in the dye adsorption capability which may shed a new light in the application of biocompost, biogas and bioelectricity production. Toxicity assessments conducted using the treated dye solution against *Vigna radiata*, *Allium cepa, Artemia salina* and *Catla catla* showed reduction in the toxic effects reinforcing its ecofriendly nature. Reuse of treated dye solution for dyeing also controls the pollution level. The outcomes derived from the physicochemical analysis of the algal compost may hold the potential to enhance both soil fertility and plant growth. The successful decolorization of

RO107 and the comprehensive understanding of the process parameters and adsorption mechanisms pave the way for innovative and sustainable approaches to address the pressing challenges of industrial wastewater pollution. This research serves as a testament to the power of bio-based materials in mitigating environmental issues and offers a promising path towards a cleaner and green environment.

References

Khan, S., Naushad, M., Govarthanan, M., Iqbal, J., and Alfadul, S. M. (2022). Emerging contaminants of high concern for the environment: Current trends and future research. Environmental Research, 207, 112609. https://doi.org/10.1016/j.envres.2021.112609.

Awwad, A., Amer, M., & Al-aqarbeh, M. (2020). TiO2-kaolinite nanocomposite prepared from the Jordanian Kaolin clay: Adsorption and thermodynamics of Pb (II) and Cd (II) ions in aqueous solution. Chemistry International. https://doi.org/10.5281/zenodo.3597558

Sarojini, G., Kannan, P., Rajamohan, N., Rajasimman, M., and Vo, D. V. N. (2023). Dyes removal from water using polymeric nanocomposites: a review. Environmental Chemistry Letters, 21(2), 1029-1058. http://dx.doi.org/10.1007/s10311-022-01547-1

Deokar, R., and Sabale, A. (2014). Biosorption of methylene blue and malachite green from binary solution onto Ulva Lactuca. Int. J. Curr. Microbiol. Appl. Sci, 3(5), 295-304.

Silva-Stenico, M. E., Vieira, F. D., Genuário, D. B., Silva, C. S., Moraes, L. A. B., and Fiore, M. F. (2012). Decolorization of textile dyes by cyanobacteria. Journal of the Brazilian Chemical Society, 23, 1863-1870.

Dönmez, G., and Aksu, Z. (2002). Removal of chromium (VI) from saline wastewaters by Dunaliella species. Process biochemistry, 38(5), 751-762.

Robson, T. O., Fowler, M. C., and Barrett, P. R. (1976). Effect of some herbicides on freshwater algae. Pesticide science, 7(4):391-402.

Kaur, B., Kumar, B., Garg, N., and Kaur, N. (2015). Statistical optimization of conditions for decolorization of synthetic dyes by *Cordyceps militaris* MTCC 3936 using RSM. BioMed research international, 2015. https://doi.org/10.1155/2015/536745

Jawad, A. H., Ishak, M. M., Farhan, A. M., and Ismail, K. (2017). Response surface methodology approach for optimization of color removal and COD reduction of methylene blue using microwave-induced NaOH activated carbon from biomass waste. Desalination and Water Treatment, 62, 208-220. http://dx.doi.org/10.5004/dwt.2017.20132

Kumar, N., Sinha, S., Mehrotra, T., Singh, R., Tandon, S., and Thakur, I. S. (2019). Biodecolorization of azo dye Acid Black 24 by *Bacillus pseudomycoides*: Process optimization using Box Behnken design model and toxicity assessment. Bioresource technology reports, 8, 100311. https://doi.org/10.1016/j.biteb.2019.100311

Vinayagam, R., Dave, N., Varadavenkatesan, T., Rajamohan, N., Sillanpää, M., Nadda, A. K., and Selvaraj, R. (2022). Artificial neural network and statistical modelling of biosorptive removal of hexavalent chromium using macroalgal spent biomass. Chemosphere, 296, 133965.

Soares, A. P. D. M. R., de Oliveira Carvalho, F., de Farias Silva, C. E., da Silva Gonçalves, A. H., and de Souza Abud, A. K. (2020). Random Forest as a promising application to predict basic-dye biosorption process using orange waste. Journal of Environmental Chemical Engineering, 8(4), 103952.

Lata, H., Garg, V. K., and Gupta, R. K. (2007). Removal of a basic dye from aqueous solution by adsorption using Parthenium hysterophorus: an agricultural waste. Dyes and pigments, 74(3), 653-658.

Abou Oualid, H., Abdellaoui, Y., Laabd, M., El Ouardi, M., Brahmi, Y., Iazza, M., & Abou Oualid, J. (2020). Eco-efficient green seaweed codium decorticatum biosorbent for textile dyes: Characterization, mechanism, recyclability, and RSM optimization. ACS omega, 5(35), 22192-22207.

Indah, S., Helard, D., & Binuwara, A. (2018). Studies on desorption and regeneration of natural pumice for iron removal from aqueous solution. Water Science and Technology, 2017(2), 509-515.

Rice, E. W., Bridgewater, L., & American Public Health Association (Eds.). (2012). Standard methods for the examination of water and wastewater (Vol. 10). Washington, DC: American public health association.

Saber, M., Mokhtar, M., Bakheit, A., Elfeky, A. M., Gameh, M., Mostafa, A. and Hamada, A. (2022). An integrated assessment approach for fossil groundwater quality and crop water requirements in the El-Kharga Oasis, Western Desert, Egypt. Journal of Hydrology: Regional Studies, 40, 101016. https://doi.org/10.1016/j.ejrh.2022.101016

Vaithiyanathan, T., and Sundaramoorthy, P. (2017) Analysis of sugar mill effluent and its influence on germination and growth of African marigold (Tagetes erecta L.). Appl Water Sci 7:4715-4723. https://doi.org/10.1007/s13201-017-0634-1

Eshghi, S., and Kashi, F. J. (2022). Bacterial synthesis of magnetic Fe3O4 nanoparticles: decolorization acid red 88 using FeNPs/Ca-Alg beads. Arabian Journal of Chemistry, 15(9), 104032.

Prasad, A. A., and Rao, K. B. (2013). Aerobic biodegradation of Azo dye by Bacillus cohnii MTCC 3616; an obligately alkaliphilic bacterium and toxicity evaluation of metabolites by different bioassay systems. Applied microbiology and biotechnology, 97, 7469-7481.

Parmar, A. I., and Shah, A. I. (2021). Acute toxicity, behavioural response and haematological alterations of Catla catla exposed to Reactive Red 120 textile dye. Indian Journal of Experimental Biology (IJEB), 59(04), 275-279.

Kandasamy, N., Kaliappan, K., and Palanisamy, T. (2021). Upcycling sawdust into colorant: Ecofriendly natural dyeing of fabrics with ultrasound assisted dye extract of Pterocarpus indicus Willd. Industrial Crops and Products, 171, 113969.

Khan, S. A., Sharma, G. K., Malla, F. A., Kumar, A., and Gupta, N. (2019). Microalgae based biofertilizers: a biorefinery approach to phycoremediate wastewater and harvest biodiesel and manure. Journal of Cleaner production, 211, 1412-1419.

El-Naggar, N. E. A., and Rabei, N. H. (2020). Bioprocessing optimization for efficient simultaneous removal of methylene blue and nickel by Gracilaria seaweed biomass. Scientific Reports, 10(1), 17439.

Jawad, A. H., Rashid, R. A., Mahmuod, R. M., Ishak, M. A. M., Kasim, N. N., and Ismail, K. (2016). Adsorption of methylene blue onto coconut (*Cocos nucifera*) leaf: optimization, isotherm and kinetic studies. Desalination and Water Treatment, 57(19), 8839-8853. https://doi.org/10.1080/19443994.2015.1026282

Wang, C., Chen, W., Yang, L., Wei, R., Ni, J., and Yang, Y. (2019). Insights into the roles of the morphological carbon structure and ash in the sorption of aromatic compounds to wood-derived biochars. Science of the total environment, 693, 133455. https://doi.org/10.1016/j.scitotenv.2019.07.261